

## Influence of starter culture on the physicochemical properties of rice bran sourdough and physical quality of sourdough bread

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### Abstract

The effect of mixed strain culture of lactic acid bacteria (LAB) and Yeast, and yeast or *L. brevis*, *L. plantarum*, or *L. sanfranciscensis* on the physicochemical properties (pH, TTA, organic acid, ethanol, and sugar content) of rice bran sourdough was investigated. Starter culture with optimum physicochemical properties was used to ferment rice and wheat bran for sourdough production. Rice and wheat bran sourdough and non-fermented rice and wheat bran were mixed with wheat flour at 10% substitution level for bread production. Results showed that rice bran fermented with *L. plantarum* had the best physicochemical properties compared to rice bran sourdough produced by other LAB or mixed culture. The specific volume of bread sample made with rice bran sourdough (4.65 cm<sup>3</sup>/g) was higher than that of the bread samples made from wheat bran sourdough (4.32 cm<sup>3</sup>/g) and non-fermented bran (3.74 – 4.24 cm<sup>3</sup>/g), but not significantly different from the control (100% wheat) bread (4.85 cm<sup>3</sup>/g). The crumb colour of the rice bran and rice bran sourdough substituted bread was lighter than that of the other bread samples. Crust colour of all the bread samples was not significantly different ( $p > 0.05$ ). At the end of 6 days storage period, bread samples from control and wheat bran sourdough were firmer than that from rice bran sourdough, however, crumb firmness values were highest in non-fermented bran substituted bread. Sensory analysis result revealed that rice bran sourdough bread was more acceptable than wheat bran sourdough bread, and non-fermented rice and wheat bran substituted bread.

## 1. Introduction

Rice bran is the by-product obtained during rice polishing or milling (Zhang *et al.*, 2012). It comprises of bran and germ, and in some cases may contain some amount of starchy endosperm. Rice bran contains moderate amount of carbohydrate (31-52%), fiber (27%) and protein (11.8-15.6%) (Chinma *et al.*, 2015). Although, the use of wheat bran is gaining increasing popularity in baked goods, there is limited usage of other cereals bran in food industries. For example, rice bran is not commercially utilized for food formulation despite its composition. It is only used in animal feed formulation.

The use of bran in bread making has been reported to weaken the structure and baking quality of dough, decrease bread volume and elasticity of the crumb and thus, reduce the overall quality of bread (Noort *et al.*, 2010). In order to improve the quality of baked goods substituted with bran, many studies have tried to improve

the quality of cereal bran by treating it with enzyme (Katina *et al.*, 2006; Sanz Penella *et al.*, 2008), subjecting bran to extrusion process (Gómez *et al.*, 2011), and through particle size reduction and fermentation (Coda *et al.*, 2014).

Generally, fermented cereal bran is not commonly used in food formulation. However, researchers have studied and reported the nutritional importance of fermented cereal bran and sourdough fermentation, and their potentials in improving the quality of bran substituted baked foods (Salmenkallio-Marttila *et al.*, 2001; Poutanen *et al.*, 2009; Katina *et al.*, 2012). Lactic acid bacteria (LAB) and yeasts have been successfully used for bran fermentation (Coda *et al.*, 2015). Rice bran fermented with yeast has been reported to contain significantly higher protein content compared to non-fermented rice bran (Chinma *et al.*, 2014).

Sourdough fermentation is one of the most recently used food biotechnology for improving sensory,

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structural, nutritional properties and shelf life of bread (Gobbetti *et al.*, 2014). During sourdough fermentation, acidification, proteolysis and enzymes activation occurs. These processes lead to biochemical changes which positively affect dough and baked good matrix and thus, enhance the nutritional and functional quality of baked goods (Poutanen *et al.*, 2009; Gobbetti *et al.*, 2014). The evaluation of sourdough fermentation is generally based on parameters such as pH, acidity, and microflora, while the quality of bread prepared with sourdough are determined by low pH and a high ratio of lactic/acetic acids, high loaf volumes and low rates of staling during storage (Gül *et al.*, 2005). Sourdough fermented by *Lactobacillus plantarum* has been reported to improve the quality of gluten-free bread (Moore *et al.*, 2008).

The use of rice bran sourdough in taftoon bread (Torkamani *et al.*, 2015) and Iranian bread (Farahmand *et al.*, 2015) has been reported. The aim of this study was to investigate the effect of using Malaysian rice bran sourdough as starter culture in pan bread, and the objectives were to determine the physicochemical properties of rice bran sourdough produced from rice bran fermented with mixed culture of LAB and yeast, and yeast or LAB, and to determine the sensory attributes and storage stability of rice bran sourdough bread. This study will provide additional information on the use of rice bran sourdough as a functional food ingredient, increase food usage of rice bran and add variety to sourdough bread.

## 2. Materials and methods

### 2.1 Materials

Rice bran (MR 219 variety) was obtained from Padiberas Nasional Berhad rice mill in Sekinchan, Selangor, Malaysia. Commercial baker's yeast (Mauripan®) was from AB Mauri Malaysia, SDN, BHD, Selangor Dahrul Elsan. Commercial wheat flour and wheat bran, sugar, salt, and shortening were purchased from a local supermarket in Serdang, Selangor, Malaysia. Rice and wheat bran were sieved using a Haver EML digital plus test sieve shaker (Harver and Boecker, 59302, OELDE, Germany) to particle size of < 300 µm. Lactic acid bacteria (*L. brevis* ATCC® 8287™, *L. plantarum* ATCC® 8014™ and *L. sanfranciscensis* ATCC® 43332™) were purchased from American Type Culture Collection (ATCC). 3M™ Petrifilm™ yeast and mold count plates was purchased from 3M Company, St. Paul, Minnesota, USA. All chemicals and reagents were of analytical grade.

### 2.2 Methods

#### 2.2.1 Chemical analyses of bran

Standard AOAC (1995) methods were used for moisture (925.08) and ash (923.03). Crude fibre content was determined using AACC (2000) method 32-10.01. Protein was determined using an automated Protein Analyzer system (Kjeltec™ 4400 Analyser Unit, Sweden) according to the AOAC (1995) Kjeldhal method (992.23). Total fat was determined with FOSS Soxtec Automated System 2050 (FOSS, Sweden) according to AOAC method 945.16 (AOAC, 1995). Carbohydrate content was calculated by difference.

#### 2.2.2 Microbial strains and growth conditions

The LAB media were prepared according to manufacturer's instruction and sterilized by autoclaving at 121°C for 15 min. *Lactobacillus brevis* (*L. brevis*) was cultured at 30°C on de Man, Rogosa and Sharpe (MRS) agars for 48 h in aerobic condition, and isolated colonies were transferred into MRS broth incubated at 30°C for 48 h in shaking flask agitated at 100 rpm. *L. plantarum* and *L. sanfranciscensis* were cultured at 37°C for 48 h at atmosphere of 5% CO<sub>2</sub>. Cultured MRS broth (1 ml) was mixed with 9 ml of sterile peptone water 0.1% (v/v) by vortexing and diluted in ten-folds using serial dilution (10<sup>-2</sup> to 10<sup>-10</sup>). Serial suspension (0.1 ml) was incubated on Plate Count Agar and the colonies were counted. The bacteria suspension (10<sup>9</sup> CFU/ml) in peptone water (0.1%) was used for bran fermentation. Yeast colony forming unit (CFU) was determined by the method described by Sullivan and Bradford (2011). Yeast (1 g) was diluted in 100 mL of 0.31 mM phosphate buffer (pH 7.2) and homogenized for 5 min using a magnetic stirrer. The solution was serially diluted (1:10), inoculated onto Petrifilm plates and the plates were incubated at 25°C for 5 days before the colonies were counted.

#### 2.2.3 Preparation of rice bran and wheat bran sourdough

Rice bran sourdough was prepared according to Katina *et al.* (2006) with some modifications. Rice bran (100 g) was mixed with distilled water (130 g), bakers' yeast (1.25 g) and lactic acid bacteria (15 ml) suspension (10<sup>9</sup> CFU/ml) in a beaker and covered with aluminum foil. The bran was allowed to ferment at 35°C for 16 h in a fermenting box (Model Fx-11, Good and Well, SDN BHD, Malaysia) to produce sourdough. Seven formulations of rice bran sourdough fermented with mixed culture of LAB (10<sup>9</sup> CFU/ml) and yeast (10<sup>7</sup> CFU/ml), *L. brevis*, *L. plantarum*, *L. sanfranciscensis* and yeast (control) were prepared as shown in Table 1, and evaluated for pH, Titratable acidity (TTA), organic acids, ethanol and sugar contents. The starter culture that produced sourdough with optimum parameters was subsequently used to ferment rice and wheat bran for bran sourdough production (as described above).

Table 1. Rice bran sourdough prepared using yeast ( $10^7$  CFU) and/or lactic acid bacteria ( $10^9$  CFU)

Rice bran sourdough	Yeast <i>Saccharomyces cerevisiae</i>	Lactic Acid Bacteria (LAB)
Yeast (control)	√	X
Yeast + <i>L. brevis</i>	√	<i>L. brevis</i>
Yeast + <i>L. plantarum</i>	√	<i>L. plantarum</i>
Yeast + <i>L. sanfranciscensis</i>	√	<i>L. sanfranciscensis</i>
<i>L. brevis</i>	X	<i>L. brevis</i>
<i>L. plantarum</i>	X	<i>L. plantarum</i>
<i>L. sanfranciscensis</i>	X	<i>L. sanfranciscensis</i>

(X) – Indicates absent.

(√) - Indicates present.

## 2.2.4 Physicochemical analyses

### 2.2.4.1 pH and total titratable acidity (TTA) of rice bran sourdough

pH and TTA were measured as described by Gül et al. (2005). Rice bran sourdough (10 g) was mixed with distilled water (100 ml) to obtain a suspension. The pH value of the suspension was measured by using a pH meter. TTA was determined by titrating the suspension against 0.1 M NaOH to a final pH value of 8.5. The TTA was expressed as the amount of NaOH (ml) used for titration.

### 2.2.4.2 Extraction of organic acids, ethanol and sugar contents of the rice bran sourdough

The extraction method described by Lefebvre et al. (2002) was adopted. Sourdough (10 g) was homogenized with distilled deionized water (60 ml) and the volume was adjusted to 100 ml. The sample was centrifuged (4000 x g, 15°C, 15 min) and the supernatant was filtered under vacuum using Whatman filter paper (0.45 µm). The filtrate (10 ml) was mixed with distilled deionized water (60 ml), 5 ml of 0.085 M Carrez I solution (potassium II hexaferrocyanate) and 5 ml of 0.25 M Carrez II solutions (zinc sulfate). The mixture was neutralized with 0.1M NaOH until pH 8.0 was reached. The volume was adjusted to 100 ml with distilled deionized water and the solution was filtered through 0.45 µm nylon membrane filter and transferred to HPLC vials. The filtrate was used for HPLC analysis of rice bran sourdough samples.

### 2.2.4.3 HPLC analysis of rice bran sourdough

Separation and quantification of rice bran sourdough sugar, lactic acid, acetic acid, and ethanol were determined according to the method described by Martínez-Anaya et al. (1993), with some modifications.

The concentration of sugars in the rice bran sourdough samples were analyzed using 1100 Series high performance liquid chromatography (Agilent, Germany). Detection was performed with a refractive index detector. Chromatographic separation was performed using ZORBAX carbohydrate analysis column (4.6 mm x 150 mm). The mobile phase was 80% acetonitrile and 20% water. The temperature of the column, flow rate, and injection volume were 30°C, 1.0 mL/min, and 20 µL, respectively.

Separation and quantification of lactic acid, acetic acid and ethanol were achieved with the same HPLC system and detector used for sugar analysis. Elution solvent was 0.0005 M H<sub>2</sub>SO<sub>4</sub> at a flow rate of 0.7 ml/min. Oven temperature was set at 40°C and 20 µl of the sample filtrate was injected into an ion exclusion Aminex HPX-87H column (7.8 mm x 300 mm). Fermentation quotient (FQ) of the rice bran sourdoughs was calculated as the molar ratio of lactate to acetate (Brandt et al., 2014).

### 2.2.5 Preparation of bran sourdough bread

Rice and wheat bran sourdough were prepared as described above (see section 2.2.3) using *L. plantarum* only. The rice and wheat bran sourdough, and non-fermented wheat and rice bran were used to substitute wheat flour at the level of 10 g per 100 g of flour.

Bran sourdough breads were produced by replacing 10% of the flour with an equivalent quantity of flour in the form of sourdough. The following five bread types were produced: 1) control bread (100% wheat flour), 2) rice bran bread (non-fermented rice bran), 3) wheat bran bread (non-fermented wheat bran), 4) rice bran sourdough bread (fermented rice bran) and, 5) wheat bran sourdough bread (fermented wheat bran). Other ingredients were wheat flour (100 g), water (63 g), dried instant yeast (1.5 g), sugar (6 g), salt (1.5 g) and shortening (5 g). The amounts of the ingredients used were based on the weight of wheat flour and wheat flour blends.

The wheat flour and other dry ingredients were mixed using a mixer (5K5SS Kitchen Aid spiral, St Joseph, Michigan). Water was added to the mixture followed by shortening. The dough was mixed at a low speed (Level 1) for 6 min followed by high speed (Level 3) mixing for another 6 min. The dough was divided into 380 g portion and each portion was molded into round shape and allowed to proof at room temperature for 10 min. The dough was then punched, rolled, placed into a greased aluminum pan (80 x 190 x 80 mm) and proofed in a BERJAYA KR 105 proofer at 39°C and 60% RH for 45 min. The baking process was carried out at 200°C for

30 min using an electric oven (Europa jet cook system, Valencia 688E, Malaysia). The bread loaves were cooled in open air at room temperature for 2 h before specific volume and texture analyses were carried out. Samples for crumb firmness were placed in zippered polypropylene bags and stored at room temperature for 6 days, crumb firmness was determined at 2 days intervals.

### 2.2.6 Bread quality assessment

#### 2.2.6.1 Specific volume, crumb and crust colour

Loaf volume and specific volume were determined by the method described by Charoenthaikij *et al.* (2012). Loaf volume was measured by using the rape seed displacement method, while specific volume was calculated as loaf volume/weight. Specific volume was expressed as  $\text{cm}^3 \text{g}^{-1}$ .

Crumb and crust colour of the bread samples were determined using a chromatometer (Konica Minolta CR300 tristimulus, Osaka, Japan) calibrated with a white reference plate ( $Y=94.1$ ;  $x=0.3154$ ;  $y=0.3314$ ). Readings were recorded as  $L^*$  (lightness),  $a^*$  (redness),  $b^*$  (yellowness).

#### 2.2.6.2 Crumb firmness

Crumb firmness was measured at 0, 2, 4 and 6 days of storage in order to assess the potential shelf life of the bread. The crumb firmness of the bread samples was determined according to AACC (74-09) standard method (AACC, 2000) using texture profile analysis test. The test was performed with a Stable Micro Systems TA-XT Plus Texture Analyzer (Godalming, England) equipped with a 36 mm diameter aluminum cylindrical probe. The bread was sliced into 25 mm thickness, and edges of the slices were cut off before measurement. The sliced bread was compressed to 40% deformation with a trigger force of 5 g at pre-test, test speed and post-test speed of 1 mm/s, 1.7 mm/s, and 10 mm/s, respectively. Firmness values were reported as the average of three measurements.

### 2.3 Sensory evaluation

The bread samples were evaluated by fifty untrained panelists, comprising of staff and students of the Faculty of Food Science and Technology, Universiti Putra Malaysia. The bread samples were scored using a 9-point hedonic scale, ranging from 1 (dislike extremely) to 9 (like extremely). Samples were evaluated for colour, flavour, texture, appearance, taste and overall acceptance.

### 2.4 Statistical analysis

Data were reported as mean values of three replicates for each sample except for sensory analysis. Statistical analysis was performed using Minitab version

16.0 software. Data obtained were subjected to one-way analysis of variance (ANOVA). Turkey's multiple comparison tests were used to determine the level of significant differences ( $P < 0.05$ ) between the samples.

## 3. Results and discussion

### 3.1 Chemical composition of bran

The chemical composition of rice and wheat bran used in this study is presented in Table 2. The results showed that the wheat bran used for the sourdough preparation had higher moisture (14.5%), fibre (14%) and carbohydrate (48%) contents compared to rice bran. However, rice bran had higher ash (8.5%) and fat contents (18%), possibly due to the presence of germ in the bran (Moongngarm *et al.*, 2012). The protein content of the wheat and rice bran was similar.

Table 2. Chemical composition of rice and wheat bran

Chemical parameters	Rice bran <sup>b</sup>	Wheat bran <sup>a,b</sup>
Moisture content (% dm)	7.2 ± 0.2	14.5 <sup>a</sup>
Protein (% dm)	11.7 ± 1.2	12.0 <sup>a</sup>
Fat (% dm)	18 ± 3.8	5.4 <sup>b</sup>
Fibre (% dm)	8.6 ± 0.5	14 <sup>a</sup>
Ash (% dm)	8.5 ± 0.2	6 <sup>a</sup>
Carbohydrate (% dm)	45.9 ± 1.7	48.1 <sup>b</sup>

<sup>a</sup> Data are based on information provided on the label.

<sup>b</sup> Data determined by author.

<sup>a,b</sup> Combination of data provided on the label and data determined by author

### 3.2 Physicochemical properties

#### 3.2.1 pH and total titratable acidity (TTA) of rice bran sourdough

The results of pH profile of the rice bran sourdough showed that there was no significant difference between the sample fermented with yeast only, and those fermented with a mixed culture of yeast and LAB, with values in the range of 5.82-5.88 (Table 3). This could be due to inhibition of yeast activities as a result of competition between LAB and yeast for sugar substrate during the fermentation process (Skinner and Leathers, 2004). However, sourdough fermented with *L. brevis*, *L. plantarum*, and *L. sanfranciscensis* had significantly ( $p < 0.05$ ) lower pH value (4.09) compared with those fermented with a combination of yeast and LAB strains. This result was in agreement with the findings of Clarke *et al.* (2002), who reported higher pH value for sourdough fermented with mixed strains starter culture. On the other hand, the pH of the *L. brevis*, *L. plantarum*, and *L. sanfranciscensis* fermented rice bran sourdough was not significantly different from each other, although bran fermented with *L. plantarum* had the lowest pH value among the sourdough samples. The pH values

(4.09-4.11) reported for *L. brevis*, *L. plantarum*, and *L. sanfranciscensis* starter culture fermented rice bran sourdough reported in this study are within the range of pH (4.0-4.3) reported for wheat dough fermented with *L. plantarum*, and *L. brevis* (Clarke et al., 2002).

The TTA of the yeast fermented sourdough was higher (11.23), but not significantly different ( $p < 0.05$ ) from that of the sourdough samples fermented with mixed starter culture strains (10.60-11.10) (Table 3). However, rice bran sourdough fermented with *L. brevis* had the highest TTA value (21.8) followed by the sourdough fermented with *L. plantarum* (21.2).

Table 3. pH and titratable acidity (TTA) of rice bran sourdoughs fermented with yeast and/or lactic acid bacteria

Sourdough	pH	TTA
Yeast (control)	5.88±0.02 <sup>a</sup>	11.23±0.29 <sup>c</sup>
Yeast + <i>L. brevis</i>	5.85±0.02 <sup>a</sup>	11.10±0.27 <sup>c</sup>
Yeast + <i>L. plantarum</i>	5.83±0.01 <sup>a</sup>	10.77±0.31 <sup>c</sup>
Yeast + <i>L. sanfranciscensis</i>	5.82±0.02 <sup>a</sup>	10.60±0.27 <sup>c</sup>
<i>L. brevis</i>	4.10±0.02 <sup>c</sup>	21.83±0.65 <sup>a</sup>
<i>L. plantarum</i>	4.09±0.01 <sup>c</sup>	21.20±0.10 <sup>ab</sup>
<i>L. sanfranciscensis</i>	4.11±0.02 <sup>c</sup>	20.60±0.17 <sup>b</sup>

The lower pH and higher TTA values recorded for rice bran sourdough fermented with *L. brevis*, *L. plantarum*, and *L. sanfranciscensis* starter culture compared with those fermented with mixed strains of LAB and yeast could be because the presence of yeast becomes negative at long fermentation period, thus, pH and TTA of dough depends mainly on LAB (Chavan and Chavan, 2011). It could also be due to competition between baker's yeast and LAB for carbohydrate source (Gurbuz et al., 2010).

### 3.2.2 Organic acids, ethanol and sugar contents of the rice bran sourdough

The organic acids determined in this study were lactic acid and acetic acid. These acids are important for flavour and taste of sourdough bread (Hansen and Hansen, 1996). The amount of lactic acid produced in the sourdough fermented with *L. brevis*, *L. plantarum*, and *L. sanfranciscensis* was higher than that in other samples (Table 4). Higher lactic acid content recorded for the sourdough fermented with *L. brevis*, *L. plantarum*, and *L. sanfranciscensis* starter culture could be ascribed to improved activities of LAB, which resulted in the rapid production of lactic acid in the sourdoughs. This result was in agreement with the findings of Gurbuz et al. (2010), who reported that LAB grows and produce lactic acid more slowly in mixtures containing yeasts than in pure culture. Sourdough with high acid will have improved proteolysis and protein

solubility. This will, in turn, lead to improve texture in bread made with bran sourdough (Katina et al., 2006). This indicates that bread made from rice bran sourdough with high to moderate acid content will have better textural properties. The trend of lactic acid values recorded for rice bran sourdough in this study was incongruent with that of Collar (1996).

Mixed culture of *L. sanfranciscensis* and yeast produced the highest amount of acetic acids in the sourdoughs (Table 4). This result is in line with the result of Vollmar and Meuser (1992), who reported that acetic acid production was optimal when *L. sanfranciscensis* was associated with *S. cerevisiae*, while fermentation with yeast did not produce the same effect. In contrary, Colla (1996) and Merseburger et al. (2005), reported that LAB grows and produce acetic acids slowly in a mixed culture of LAB and yeast. Acetic acid has an antimicrobial effect on rope-producing *Bacillus* and anti-mould effect on sourdough bread (Rosenquist and Hansen, 1998).

Sourdough fermented with yeast, and mixed culture had higher concentrations of ethanol compared with those fermented with *L. brevis*, *L. plantarum*, and *L. sanfranciscensis* (Table 4). This could be due to the effect of yeast fermentation on carbohydrate, in which glucose is converted into ethanol (Gurbuz et al., 2010). This result is in agreement with the previous report on headspace flavour profile of dough, in which ethanol is the most prominent compound in dough fermented with yeast while diacetyl is prominent in dough fermented with LAB (Colla, 1996). Sourdough fermented with *L. plantarum* had the highest fermentation quotient (FQ) value (3.88) compared with the other samples (Table 4). This value was close to the recommended FQ value (4.00) for sourdough (Spicher and Stephen, 1999).

The soluble carbohydrate contents of rice bran sourdough; glucose, maltose, fructose and sucrose ranged from 0.35-1.11 g/100g, 0.48-1.06 g/100g, 0.72-1.34 g/100g and 0.55-1.32 g/100g, respectively (Table 5). Rice bran sourdough fermented with yeast (control sample) had the highest glucose (1.1 g/100g) and maltose content (1.06 g/100g) compared to the sourdoughs fermented with mixed culture or *L. brevis*, *L. plantarum*, and *L. sanfranciscensis*. This could be because yeasts and LAB have different kinetics for soluble carbohydrate uptake (Gurbuz et al., 2010). This observation was in agreement with the report of Colla et al. (1996). The fructose content of all the sourdough samples was not significantly different ( $p > 0.05$ ). This is possibly because both yeast and LAB have similar kinetics for fructose uptake and fermentation (Koca et al., 2002; Gurbuz, 2010). There were no significant

Table 4. Organic acids and ethanol contents of rice bran sourdoughs fermented with yeast and/or lactic acid bacteria

Rice bran sourdoughs	Concentration (g/100g)			Fermentation Quotient (FQ)
	Lactic acid	Acetic acid	Ethanol	
Yeast (control)	0.13±0.01 <sup>b</sup>	0.12±0.05 <sup>c</sup>	0.95±0.14 <sup>ab</sup>	1.08
Yeast + <i>L. brevis</i>	0.07±0.00 <sup>c</sup>	0.05±0.00 <sup>d</sup>	1.17±0.09 <sup>a</sup>	-
Yeast + <i>L. plantarum</i>	0.14±0.01 <sup>b</sup>	0.19±0.03 <sup>b</sup>	1.01±0.19 <sup>a</sup>	0.74
Yeast + <i>L. sanfranciscensis</i>	0.11±0.00 <sup>b</sup>	0.28±0.02 <sup>a</sup>	0.93±0.08 <sup>ab</sup>	0.39
<i>L. brevis</i>	0.32±0.03 <sup>a</sup>	n.d	0.40±0.11 <sup>cd</sup>	-
<i>L. plantarum</i>	0.31±0.03 <sup>a</sup>	0.08±0.02 <sup>d</sup>	0.44±0.08 <sup>cd</sup>	3.88
<i>L. sanfranciscensis</i>	0.29±0.06 <sup>a</sup>	n.d	0.74±0.19 <sup>bc</sup>	-

Values in the same column with different superscript letters are significantly different ( $p < 0.05$ ).

n.d – indicates not detected.

Values are means ± standard deviations of triplicate analyses.

Table 5. Sugar contents of rice bran sourdoughs (g/100g) fermented with yeast and/or lactic acid bacteria

Rice Bran Sourdoughs	Glucose	Maltose	Fructose	Sucrose
Yeast (control)	1.11±0.05 <sup>a</sup>	1.06±0.16 <sup>c</sup>	0.72±0.01 <sup>c</sup>	0.54±0.03 <sup>g</sup>
Yeast + <i>L. brevis</i>	1.02± 0.04 <sup>a</sup>	1.00±0.05 <sup>c</sup>	0.80±0.02 <sup>c</sup>	0.69±0.01 <sup>g</sup>
Yeast + <i>L. plantarum</i>	0.93±0.03 <sup>a</sup>	0.82±0.03 <sup>c</sup>	1.04±0.02 <sup>c</sup>	0.67±0.01 <sup>g</sup>
Yeast + <i>L. sanfranciscensis</i>	0.85±0.02 <sup>a</sup>	0.79±0.01 <sup>c</sup>	0.94±0.04 <sup>c</sup>	0.74±0.05 <sup>g</sup>
<i>L. brevis</i>	0.43±0.05 <sup>b</sup>	0.54±0.04 <sup>d</sup>	0.95±0.02 <sup>c</sup>	1.32±0.08 <sup>f</sup>
<i>L. plantarum</i>	0.38±0.06 <sup>b</sup>	0.48±0.01 <sup>d</sup>	1.34±0.05 <sup>c</sup>	1.07±0.06 <sup>f</sup>
<i>L. sanfranciscensis</i>	0.35±0.03 <sup>b</sup>	0.52±0.05 <sup>d</sup>	0.89±0.05 <sup>c</sup>	0.93±0.04 <sup>f</sup>

Values are means ± standard deviations of triplicate analyses.

Mean values in the same column with different superscript letters are significantly different ( $p < 0.05$ ).

Table 6. Specific volume, crumb and crust colour of rice and wheat bran supplemented breads

Bread sample	Specific Volume (cm <sup>3</sup> /g)	Crumb			Crust		
		L*	a*	b*	L*	a*	b*
Control (no bran)	4.85 ± 0.17 <sup>f</sup>	68.34 ±	-0.87 ±	8.02±	45.37 ±	14.85 ±	25.75 ±
		1.03 <sup>ab</sup>	0.10 <sup>d</sup>	0.86 <sup>f</sup>	3.23 <sup>g</sup>	0.97 <sup>h</sup>	4.07 <sup>i</sup>
Rice bran	4.24 ± 0.07 <sup>g</sup>	70.64 ±	-0.77 ±	12.39 ±	47.81 ±	14.20 ±	27.74 ±
		0.20 <sup>a</sup>	0.08 <sup>d</sup>	0.60 <sup>e</sup>	3.94 <sup>g</sup>	0.48 <sup>h</sup>	2.96 <sup>i</sup>
Wheat bran	3.74 ± 0.12 <sup>h</sup>	67.82 ±	1.21 ±	13.98 ±	44.04 ±	13.25 ±	23.53 ±
		1.77 <sup>ab</sup>	0.22 <sup>c</sup>	0.47 <sup>e</sup>	3.32 <sup>g</sup>	0.84 <sup>h</sup>	3.68 <sup>i</sup>
Rice bran sourdough	4.65 ± 0.13 <sup>f</sup>	70.10 ±	-0.87 ±	12.41 ±	45.92 ±	14.80 ±	26.78 ±
		3.11 <sup>a</sup>	0.14 <sup>d</sup>	1.52 <sup>e</sup>	1.47 <sup>g</sup>	0.02 <sup>h</sup>	2.96 <sup>i</sup>
Wheat bran sourdough	4.32 ± 0.03 <sup>g</sup>	64.45 ±	1.44 ±	12.19 ±	45.20 ±	14.19 ±	25.03 ±
		1.04 <sup>b</sup>	0.06 <sup>c</sup>	0.31 <sup>e</sup>	1.72 <sup>g</sup>	0.53 <sup>h</sup>	1.91 <sup>i</sup>

Values are means ± standard deviations of triplicate analyses.

Mean values in the same column with different superscript letters are significantly different ( $p < 0.05$ ).

Table 7. Sensory analysis of Rice and wheat bran sourdough bread

Quality parameters	Wheat flour (control)	Rice bran	Wheat bran	Rice bran sourdough	Wheat bran sourdough
Colour	7.24±0.05 <sup>a</sup>	7.09±0.07 <sup>b</sup>	6.87±0.04 <sup>c</sup>	7.36±0.18 <sup>a</sup>	6.82±0.05 <sup>c</sup>
Flavour	7.37±0.11 <sup>a</sup>	6.02±0.09 <sup>d</sup>	6.55±0.04 <sup>c</sup>	6.62±0.11 <sup>bc</sup>	6.78±0.03 <sup>b</sup>
Texture	7.19±0.05 <sup>a</sup>	6.59±0.03 <sup>c</sup>	6.12±0.03 <sup>d</sup>	7.11±0.05 <sup>a</sup>	6.72±0.02 <sup>b</sup>
Appearance	7.36±0.10 <sup>a</sup>	7.19±0.03 <sup>b</sup>	7.12±0.03 <sup>b</sup>	7.37±0.04 <sup>a</sup>	7.25±0.04 <sup>ab</sup>
Taste	7.30±0.05 <sup>a</sup>	6.63±0.09 <sup>b</sup>	6.55±0.09 <sup>b</sup>	7.24±0.03 <sup>a</sup>	7.17±0.05 <sup>a</sup>
Overall acceptability	7.24±0.05 <sup>a</sup>	6.43±0.02 <sup>c</sup>	6.39±0.02 <sup>c</sup>	7.25±0.02 <sup>a</sup>	6.94±0.07 <sup>b</sup>

Values are mean ± standard deviation (n = 50).

Mean values along the same row with different alphabet (s) are significantly different ( $p < 0.05$ ).

differences ( $p > 0.05$ ) in the sucrose content of the control sourdough and the sourdough produced from a mixed culture, however, significant differences ( $p < 0.05$ ) existed between the sucrose contents of mixed culture and *L. brevis*, *L. plantarum*, and *L. sanfranciscensis* fermented sourdoughs. This could be due to invertase hydrolysis of sucrose into glucose and fructose by yeast (Aksu and Kutsal, 1986), which leads to rapid sucrose depletion in the sourdough fermented with mixed culture and the control sample (Martínez -Anaya, 1996).

Among the starter cultures, *L. plantarum* proved to be the most effective for rice bran sourdough production in terms of acidity (pH and TTA) and fermentation quotient, which are important parameters for assessing sourdough quality. Thus, *L. plantarum* was subsequently used to ferment rice and wheat bran for sourdough bread production.

### 3.3 Bread quality

#### 3.3.1 Specific volume, crumb and crust colour

Bread samples substituted with fermented and non-fermented rice and wheat bran had reduced specific volume when compared with 100% wheat bread (control) (Table 6). However, the specific volume of rice ( $4.65 \text{ cm}^3/\text{g}$ ) and wheat bran sourdough bread ( $4.32 \text{ cm}^3/\text{g}$ ) was significantly higher than that of rice ( $4.24 \text{ cm}^3/\text{g}$ ) and wheat bran ( $3.74 \text{ cm}^3/\text{g}$ ) bread. The specific volume of rice bran sourdough bread was not significantly different ( $p > 0.05$ ) from that of the control bread (Table 6), while the specific volume of the wheat bran sourdough bread was significantly ( $p < 0.05$ ) lower than the control (Table 6). In contrast to this result, Corsetti *et al.* (2000), reported higher volume for bread produced with LAB and *S. cerevisiae* association than that of bread produced with *S. cerevisiae* alone. The differences in the specific volume of the sourdough substituted bread reported in this study could be due to differences in the fibre content of the bran (Table 2). Higher specific volume recorded for bran sourdough substituted bread compared to non-fermented bran substituted bread samples could be due to increased retention of  $\text{CO}_2$  in the bread dough as a result of LAB acidification, which enhanced the capacity of gluten to retain  $\text{CO}_2$  (Gobbetti *et al.*, 1995). This indicates that rice bran sourdough does not have a negative effect on the bread volume. In line with this observation, Salmenkallio-Marttila *et al.* (2001) also reported that the use of fermented cereal bran in bread making has a positive influence on bread volume.

As shown in Table 6, the addition of cereal bran to wheat flour darkens the colour of the wheat bran bread crumb. However, rice bran sourdough bread had the highest crumb lightness ( $L^*$ ) value followed by that of

the rice bran bread. According to Gallagher *et al.* (2003), rice-based bread tends to have a lighter colour than wheat bread. The light colour of rice bran might be due to the presence of bound phenolics in rice (Min *et al.*, 2012). The lower  $L^*$  values recorded for wheat bran bread crumb could possibly be due to the effect of Maillard browning (Kent and Evers, 1994). Negative redness ( $a^*$ ) values recorded for the control and the rice bran substituted bread samples indicates that the colour of the bread crumb was tending towards green than red colour. This could be due to the influence of the various ingredients on the bread crumb. However, bread substituted with wheat bran had positive redness ( $a^*$ ) value. The presence of carotenoid pigments in wheat bran could be responsible for the redness of the wheat bran bread crumb (Toufeili *et al.*, 1999). There were no significant differences ( $p > 0.05$ ) in the crust colour of all the five bread samples in terms of lightness ( $L^*$ ), redness ( $a^*$ ) and yellowness ( $b^*$ ).

#### 3.3.2 Crumb firmness

Substitution of wheat flour with non-fermented rice and wheat bran resulted in increased bread crumb firmness, whereas, substitution with rice and wheat bran sourdough reduced crumb firmness. The addition of rice and wheat bran sourdough to wheat flour was effective in improving crumb texture of the bread samples (Figure 1). Changes in the firmness of the crumb after storage could be due to different staling processes occurring in the bread samples. Among the fresh (0 day storage) bread samples, wheat bran bread had the hardest crumb followed by rice bran bread. The mean crumb hardness of rice and wheat bran sourdough bread was similar to that of the control bread but lower than that of the non-fermented bran substituted bread (Figure 1). This result is also confirmed by the higher specific volume of sourdough bread samples than the bran substituted breads (Table 6). Higher crumb hardness recorded for the non-fermented bran substituted breads could be

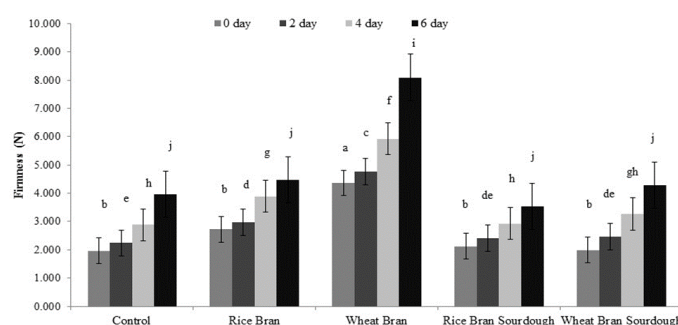


Figure 1. Crumb firmness values of bread loaves produced from 100% wheat flour (control), wheat flour supplemented with unfermented rice and wheat bran, and rice and wheat bran sourdough during a 6 days storage period. Bars with different alphabets within the different bread types are significantly different ( $P < 0.05$ ).

probably due to the high fibre content of rice and wheat bran (Table 2) and thus, higher water absorption capacity.

After 2 days of storage, rice bran sourdough bread was significantly softer than the control bread. This could be because sourdough fermentation plays important role in preserving bread freshness by influencing the loaf moisture redistribution during storage, through acidification development (Corsetti *et al.*, 2000). The protein solubility and proteolysis enhancement during sourdough fermentation process could also modify the gluten network, thereby, affecting the texture of bread (Armero and Collar, 1997).

After 6 days of storage, control bread was firmer than rice bran sourdough bread. Indicating that rice bran sourdough bread maintained its freshness during the 6 days period of storage. This could be due to the inhibitory effect of sourdough on bread staling. Many studies have reported the ability of sourdough in delaying staling process in wheat and other gluten bread (Barber *et al.*, 1992; Corsetti *et al.*, 1998; Corsetti *et al.*, 2000). Moore *et al.* (2008), also reported that *L. plantarum* can be used to improve the quality and shelf life of bread. However, bread firmness does not only depend on starter culture but also depends on other physicochemical factors (Corsetti *et al.*, 1998).

### 3.4 Sensory analysis

The mean sensory scores of the bread samples are presented in Table 7. The sensory analysis result showed that all the sensory attributes were lowest for non-fermented bran substituted bread compared to the bran sourdough bread. The highest score for almost all the sensory attributes was observed in rice bran sourdough bread and control (Table 7). The darker colour of the wheat bran and wheat bran sourdough substituted bread could be due to the inherent colour of wheat bran. The sensory scores for the colour of the bread were in conformity with that of the instrumental analysis (Table 6). Reduced sensory score recorded for the flavour of rice bran and rice bran sourdough bread might be attributed to the unfamiliarity of the panelist with rice bran odour. Generally, rice bran sourdough bread was more acceptable than rice and wheat bran substituted bread, and wheat bran sourdough bread in terms of colour, texture, appearance, taste and overall acceptability.

## 4. Conclusion

The results of this study showed that rice bran sourdough produced by *Lactobacillus plantarum* had low pH, moderate sugar contents, high TTA, and

fermentation quotient compared to sourdough fermented with a mixed culture of yeast and LAB or a single strain of yeast/other LAB. Rice bran sourdough substituted bread had higher specific volume, longer shelf life and best sensorial scores for colour, appearance and overall acceptability than the control bread and other bran and sourdough substituted bread. This study has demonstrated the potential of *L. plantarum* fermented rice bran sourdough for sourdough bread production. The use of rice bran sourdough for bread making would add value to rice bran and increase its utilization in rice-producing countries, and also provide additional income for rice milling industries.

## Conflict of Interest

Authors have no conflict of interest.

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